eag domain-containing fragments (N1-135, N1-354) were demonstrated to reduce relative outward current, slow deactivation, and slow recovery from inactivation, resulting in channels with properties similar to those measured in wild-type hERG. The proximal N-terminal region was shown to be involved in steady-state activation.

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Interactions between KCNQ1 and hERG α-Subunits are Mediated by C-Termini and Modulated by Intracellular cAMP

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KCNQ1 and hERG encode the voltage-gated K^+ channel α-subunits of the cardiac repolarizing currents I_{Ks} and I_{Kr}, respectively. These currents function *in vivo* with some redundancy to maintain appropriate action potential durations (APDs), following the concept of repolarization reserve, and mutations can manifest clinically as long QT syndrome, arrhythmia, and sudden cardiac death. Previous work in transgenic rabbit cardiomyocytes and heterologous cells demonstrated functional downregulation of complementary repolarizing currents due to direct interactions between hERG and KCNQ1 α-subunits, which may involve the hERG cyclic nucleotide binding domain (CNBD). We hypothesize that direct interactions between KCNQ1 and hERG are mediated by their C-termini and are dynamically regulated by physiological stimuli, specifically intracellular cAMP levels. We created ion channel fusions to GFP variants, which have been characterized by cellular electrophysiology and fluorescent imaging. Acceptor photobleach FRET (apFRET) was employed in fixed cells to quantitatively assess the extent of interactions based on fluorophore location and to examine the effects of altering cAMP levels. apFRET results show interactions between α-subunits with the highest FRET efficiency (E_f) for C-terminally labeled KCNQ1 and hERG (12.0 ± 5.2%), similar to intra-tetramer FRET, only between labeled hERG α-subunits (11.9 ± 5.9%). Acute treatment for 5 or 30 min. with Forskolin (100μM) + IBMX (100μM) significantly and specifically reduced E_f (7.1 ± 7.2% and 7.9 ± 4.3%, respectively, $P < 0.001$). Cells expressing a FRET positive control (KCNQ1-CFP-YFP) showed no change in E_f with treatment (21.4 ± 5.1% vs. 20.8 ± 5.6%, P=0.6). This work demonstrates regulated interactions between the C- termini of KCNQ1 and hERG, members of two distinct potassium channel families, and future studies will determine whether direct binding of cAMP abrogates KCNQ1-hERG interactions or if other downstream (PKA-mediated) processes are involved.

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Properties and Pharmacology of KCNQ1-HERG Channel Complexes

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Evidence has emerged that KCNQ1 and HERG potassium channels can directly interact when co-expressed in heterologous cells, but the reliance on double transient transfection in these prior experiments confounds interpretation of the resultant channel properties. We employed a new, highly stable HERG cell line to revisit the biophysical and pharmacological properties of KCNQ1-HERG currents. Whole-cell current was measured at the end of a 2 s pulse from −80 to +60 mV and at peak tail current (TC) recorded at −50 mV. The peak current for stable HERG cells at +50 mV was 5.5 pA/pF (+50mV) and TC amplitude was 16.9 pA/pF with activation V½ of 9.3 ± 1.9 mV (n=12). Transient transfection of stable HERG cells with KCNQ1 (0.3μg cDNA) generated 14-fold greater current density measured at +50 mV (76.6 pA/pF) and a 1.5-fold greater TC density (25.3 pA/pF) compared with HERG alone. Compared to transient expression of KCNQ1 alone, current density in HERG-KCNQ1 cells was 2.8-fold and 28-fold greater at +50 mV and for peak TC, respectively. Gating kinetics more closely resembled KCNQ1 alone as did activation V½ values (HERG, 9.3 ± 1.9 mV; KCNQ1, −14.5 ± 1.9 mV; HERG-KCNQ1, −6.8 ± 0.9 mV). Dofetilide (1μM) and HMR-1556 (10μM) were used to dissect HERG-KCNQ1 current. KCNQ1 is blocked by HMR-1556 (100% activation and 70% TC) but is dofetilide-insensitive. HERG TC is blocked 90% by dofetilide but only 30% by HMR-1556. HERG-KCNQ1 block was different: HMR-1556 reduced activation by 90% and TC by 70% and dofetilide blocked both activation (50%) and tail-currents (70%). HMR-1556 sensitive and dofetilide sensitive current components differed from KCNQ1 or HERG alone. Moreover, dofetilide sensitive current exhibit distinct gating properties from either KCNQ1 or HERG. These results demonstrate that co-expression of KCNQ1 and HERG yields current with gating and pharmacological properties distinct from the two parent channels.

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Relative Contributions of PI(4)P Pools of the Plasma Membrane and the Golgi for Maintaining the PI(4,5)P2 of the Plasma Membrane

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Plasma membrane (PM) phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) is required for KCNQ2/3 channel activity. To determine precursor sources of the plasma membrane (PM) PI(4,5)P2 pool in tsA-201 cells, we monitored KCNQ tail currents and YFP-PH(PLC-delta) translocation as real-time indicators of PM PI(4,5)P2, and GFP-OSH1-PH as an indicator of Golgi PI(4)P. We selectively depleted PI(4)P pools at the PM, or the Golgi, or both using rapamycin-translocatable enzymes, pseudojanin, an engineered tandem 4- and 5-phosphatase (SAC1 and INPP5E). Selectively depleting PI(4)P at the PM with pseudojanin-SAC (PJ-SAC; only SAC1 is active) results in a secondary decrease of PI(4,5)P2 measured by KCNQ channels or by PH-PLC domains. Compared to control pseudojanin (PJ), the decrease in current with PJ-SAC is only partial (~60% vs ~95%) and slower (140-s vs 14-s). Likewise, translocation of PH-PLC is slower (61-s vs 20-s) than with control PJ and smaller (~60% that of PJ). Depleting PI(4)P at the Golgi with PJ-SAC also induces a secondary decline of PM PI(4,5)P2 measured by KCNQ channels. The decrease is partial (35%), smaller than with PJ at the PM, and slower (60 s). Depleting PI(4)P simultaneously at the Golgi and PM with PJ-SAC recruited to both membranes also induces a slow secondary decrease of PI(4,5)P2 measured by KCNQ channels (100-s, 75%). Recruiting the ER towards the Golgi using rapamycin-induced dimerization, mimics the effects of depleting PI(4)P at the Golgi. In conclusion, the PM pool of PI(4,5)P2 depends on precursor pools of PI(4)P both in the PM and in the Golgi. The decrease in PM PI(4,5)P2 when SAC1 is active at the Golgi suggests that the Golgi contribution is on-going and does not wait until the PM is depleted. (NIH grants NS08174, GM83913 and RR025429).

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Does PI(4,5)P2 Regulate Voltage-Gated Potassium Channels?

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Phosphatidylinositol −4,5-bisphosphate (PI(4,5)P2) is an important regulator of activity of a variety of ion channels including inwardly rectifying potassium channels, KCNQ, TRP, and voltage-gated calcium channels. Several groups have provided evidence that also voltage-gated potassium (Kv) channels might be regulated by PI(4,5)P2. Taking the wide expression of Kv channels in a variety of different tissues into account, such regulation of activity by PI(4,5)P2 could potentially be of great physiological importance. To determine whether several Kv channels show a regulation of their activity by PI(4,5)P2 we have coexpressed them in tsA201-cells with either a voltage-gated lipid 5-phosphatase (VSP), a G-protein coupled receptor (M1R), or an engineered fusion protein carrying both a 4- and 5-lipid phosphatase activity (Pseudojanin, PJ). These tools deplete PI(4,5)P2 with application of depolarization, muscarinic agonists, or rapamycin, respectively. We monitored PI(4,5)P2 amounts at the plasma membrane by FRET with PH-probes from PLCδ1 simultaneously with whole-cell patch-clamp recordings. Activating VSP or PJ inhibited KCNQ2/3 channel current leaving only about 5-10 % remaining current. Activation of M1R inhibited KCNQ2/3 current a comparable amount. Thus the tools used for our assays are working. We tested with them for potential regulation of activity by PI(4,5)P2 of Kv1.1/Kvβ1.1, Kv1.3, Kv1.4 and Kv1.5/Kvβ1.3, Kv3.4, Kv4.2, Kv4.3 (both with different KChIPs and DPP6-s) and HERG/KCNE2. Interestingly, we found a substantial upregulation of current density and a removal of inactivation for Kv1.1/Kvβ1.1 and Kv3.4 upon activation of M1R, but no changes in activity upon only activating VSP or PJ. All other channels tested showed no alteration in activity in any of the assays we used. In conclusion, ''physiological'' depletion of PI(4,5)P2 at the plasma membrane does not seem to influence activity of most tested Kv channels. Supported by NIH grant NS08174 and the AvH Foundation.

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The Role of PIP2 in the Voltage-Dependent Activation of Kv7.1

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Phosphatidylinositol 4,5-bisphosphate (PIP2) is an important regulator of ion channel activity. While PIP2 modulation of voltage-independent Kir channels has been extensively studied, the mechanism of how PIP2 potentiates the activity of voltage-dependent ion channels remains uknown. In this work, we study